

Supplementary Material

Changes of cadmium storage forms and isotope ratios in rice during grain filling

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Table_S 1: Quality assessment

sample type	description	element	reference concentration		measured concentration ^a			recovery digestion ^b
			[mg (kg dry weight) ⁻¹]		mean	±sd ^d	n ^c	% ^b
rice shoot ^h	internal ISTERre rice standard		-	-	24.6	1.19	9	-
white cabbage	BCR-679	Cd ^h	1.66	0.07	1.77	0.05	3	107
soil	NIST SRM 2711		41.7	-	38.2	0.32	2	92
rice shoot ^h	internal ISTERre rice standard		-	-	35.9	1.84	9	-
white cabbage	BCR-679	Zn	79.70		92.36	2.06	3	116
soil	NIST SRM 2711		350		345	6.00	2	98
rice shoot ^h	internal ISTERre rice standard		-	-	6.48	0.46	8	-
white cabbage	BCR-679	Cu	2.89		2.93	0.20	3	101
soil	NIST SRM 2711		114		104	2.01	2	91
rice shoot ^h	internal ISTERre rice standard		-	-	1.41	0.06	8	-
white cabbage	BCR-679	Ni	27.0		30.34	1.73	3	113
soil	NIST SRM 2711		20.6		18.2	0.23	2	88
rice shoot ^h	internal ISTERre rice standard		-	-	232	9.05	8	-
white cabbage	BCR-679	Mn	13.3		15.52	0.34	3	117
soil	NIST SRM 2711		638		659	14.7	2	103

^adata from Wiggerhauser et al. 2020

^bequal to [measured concentration] / [reference concentration] *100

^cnumber of processing replicates (individually digested, purified for isotope analysis, and measured)

^dsd = standard deviation of the mean

Table_S 2: Dry weight and micro nutrients root and shoot

compartment	period/growth stage	DW		Zn				Mn				Ni				Cu			
		dry weight		concentration		mass		concentration		mass		concentration		mass		concentration		mass	
		g	±sd ^a	µg g ⁻¹	±sd ^a	µg	±sd ^a	µg g ⁻¹	±sd ^a	µg	±sd ^a	µg g ⁻¹	±sd ^a	µg	±sd ^a	µg g ⁻¹	±sd ^a	µg	±sd ^a
whole plant	flowering	25.3	0.66 B	43.5	6.97 A	1101	186 A	209	21.1 A	5284	620 B	2.57	0.81 A	64.9	20.2 A	10.9	1.85 A	277	50.2 A
	maturity	45.0	3.22 A	44.1	2.89 A	1979	88.4 A	206	11.2 A	9291	1158 A	1.90	0.20 A	85.7	12.8 A	6.93	0.64 B	311	22.0 A
	grain filling ^h	19.69	3.29	0.62	7.55	878	206	-2.56	23.9	4007	1314	-0.67	0.8	20.7	23.9	-4.01	2.0	33.6	54.9
root	flowering	4.55	0.70 b	76.1	9.31 a	351	92.5 a	87.5	25.2 a	407	165 a	10.1	2.61 a	47.3	18.1 a	35.1	3.43 a	161	38.1 a
	maturity	6.55	2.08 a	67.8	7.92 a	433	81.8 a	88.8	18.4 a	606	328 a	8.16	2.21 a	52.2	15.1 a	24.8	3.89 b	157	28.0 a
	grain filling ^h	2.00	2.2	-8.26	12.2	81.7	123	1.33	31.2	199	367	-1.99	3.42	4.85	23.6	-10.36	5.19	-4.09	47.3
shoot	flowering	20.8	0.74 b	36.2	5.26 a	750	97.0 b	235	27.3 a	4876	549 b	0.85	0.13 a	17.6	2.27 b	5.59	0.67 a	116	12.9 a
	maturity	38.4	1.48 a	40.3	3.42 a	1546	106 a	226	16.1 a	8685	836 a	0.87	0.15 a	33.5	6.64 a	4.01	0.59 b	154	20.0 a
	grain filling ^h	17.7	1.66	4.04	6.27	796	143	-9.46	31.7	3809	1000	0.02	0.20	15.9	7.02	-1.58	0.89	37.7	23.7
nodes	flowering	NA	NA	378	103	NA	NA	519	26.8	NA	NA	1.65	0.32	NA	NA	13.17	2.74	NA	NA
	maturity	NA	NA	813	315	NA	NA	428	37.3	NA	NA	1.51	0.10	NA	NA	18.81	0.90	NA	NA
	grain filling ^h	NA	NA	435	166	NA	NA	-91	23	NA	NA	-0.14	0.17	NA	NA	5.64	1.44	NA	NA
grain	harvest	4.83	1.01	26.9	1.24	130	25.6	26.1	5.3	125	29.9	1.60	0.35	7.75	2.35	4.78	0.69	23.01	5.70

^asd = standard deviation of the mean of n = 4 experimental replicates, except for the nodes (pooled to n = 2 replicates (error bar = 2sd) to obtain sufficient material for all analysis). Letters indicate significant differences of the mean (upper case = whole plant, lower case italic = root, lower case regular = shoot) determined Welch's t-test or Wilcoxon rank sum test. n: calculation as described in equation 4-6.

Table_S 3: Cadmium concentration, mass balance, Cd isotopes

compartment	period/growth stage	Cd concentration		Cd mass			Cd isotopes		
		$\mu\text{g g}^{-1}$		μg^c			$\delta^{114/110}\text{Cd} [\text{‰}]$		
		mean	$\pm\text{sd}^a$	mean	$\pm\text{sd}^a$		mean	$\pm\text{sd}^a$	
whole plant	flowering	151	24.9 A	3828	709 A		-0.45	0.01	A
	maturity	75.0	11.0 B	3380	590 A		-0.39	0.05	A
	grain filling ^b	-75.90	27.3	-448	922		0.06	0.05	
root	flowering	706	74.6 a	3214	628 a		-0.47	0.01	a
	maturity	420	66.5 b	2679	543 a		-0.45	0.07	a
	grain filling ^b	-285	99.9	-536	831		0.03	0.07	
shoot	flowering	29.6	4.61 a	614	91.6 a		-0.31	0.03	b
	maturity	18.3	2.43 b	701	84.6 a		-0.17	0.02	a
	grain filling ^b	-11.3	5.21	87.5	125		0.14	0.04	
nodes	flowering	21.2	0.30 NA	NA	NA NA		0.33	0.00	NA
	maturity	54.0	18.0 NA	NA	NA NA		0.52	0.12	NA
	grain filling ^b	32.9	9.03	NA	NA		0.18	0.06	
grain	harvest	0.98	0.05	4.73	1.03		0.49	0.01	

^asd = standard deviation of the mean of $n = 4$ experimental replicates, except for the nodes (pooled to $n = 2$ replicates (error bar = 2sd) to obtain sufficient material for all analysis). Letters indicate significant differences of the mean (upper case = whole plant, lower case italic = root, lower case regular = shoot) determined using a Welch's t -test or Wilcoxon rank sum test (whole plant isotope ratios, Cd mass shoot).
^bbalance calculation as described in equations 4-5.
^cCd mass is given as $\mu\text{g (pot)}^{-1}$; one pot contains 1.5kg and 22500 $\mu\text{g Cd}$

Table_S 4: Soil chemical parameters at flowering and maturity

period/ growth stage	rhizon pH ^b			rhizon Eh ^b			Ca(NO ₃) ₂ soil extractable Cd						
							[mg (kg soil) ⁻¹]			$\delta^{114/110}\text{Cd} [\text{‰}]$			
	%	mean	$\pm\text{sd}$	n	mean	$\pm\text{sd}$	n	mean	$\pm\text{sd}$	n	mean	$\pm 2\text{sd}$	n
vegetative ^a	4.52	0.67	10 ^c	235	45.5	10 ^c							
grain filling	4.98	0.23	2 ^c	233	23.2	2 ^c							
flowering	5.52	0.24	3	195	22.3	3	3.52	2.10	2	-0.18	0.04	2	
maturity	5.14	0.11	4	217	5.1	4	3.65	0.23	2	-0.07	0.04	2	

^aData obtained in Wiggerhauser et al. (2020)

^bsoil pH determined using rhizon samplers (see methods)

^cn = sampling time points during the growth period

Table_S 5: Dry weight, Cd concentration, Cd mass, Cd isotopes in the upper shoot at maturity

compartment	dry weight		Cd concentration		Cd mass		Cd isotopes	
	g		$\mu\text{g g}^{-1}$		μg^{b}		$\delta^{114/110}\text{Cd} [\text{‰}]$	
	mean	$\pm\text{sd}^{\text{a}}$	mean	$\pm\text{sd}^{\text{a}}$	mean	$\pm\text{sd}^{\text{a}}$	mean	$\pm\text{sd}^{\text{a}}$
shoot	38.4	1.48	18.3	2.43	701	84.6	-0.17	0.02
straw	25.6	0.96	25.5	3.02	676	84.3	-0.20	0.02
flag leaves	1.51	1.14 b	1.35	0.25 b	1.88	1.17 c	-0.01	0.05 c
panicle	2.81	0.69 a	6.52	1.24 a	18.3	6.03 a	0.41	0.04 b
grains	4.83	1.01 c	0.98	0.05 c	4.73	1.03 b	0.49	0.01 a
average/sum	9.14	1.68	2.95	3.10	24.9	6.23	0.39	0.03

^asd = standard deviation of the mean of $n = 4$ experimental replicates. For the grain filling balance calculations (italic), the sd was calculated using error propagation as described in equation 4-5. Letters indicate significant differences of the mean determined using ANOVA for Cd mass fraction

^bCd mass is given as $\mu\text{g (pot)}^{-1}$, one pot contains 1.5kg and 22500 $\mu\text{g Cd}$

Table_S 6: Results of linear combination fits of XANES and EXAFS spectra for the soils and plant parts

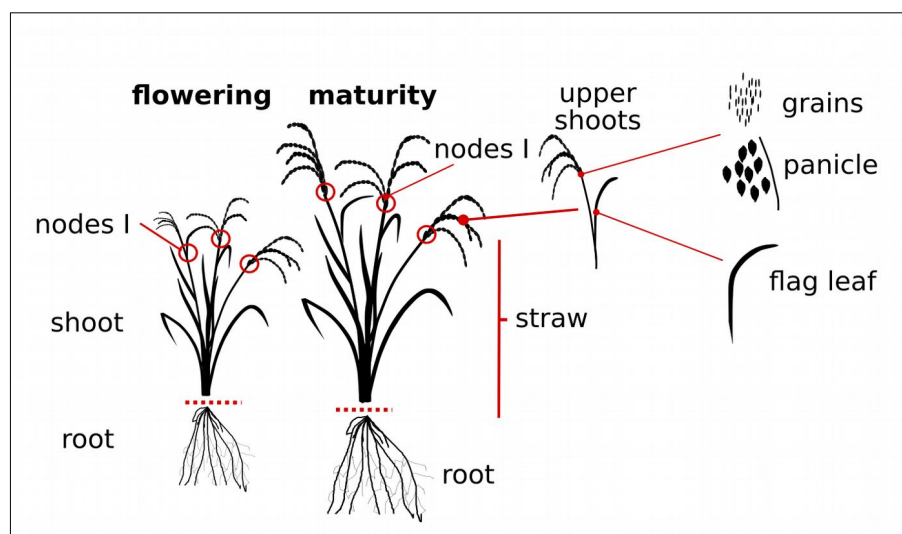
Sample ID + fit				Percentages of Cd species			Percentages normalized to 100%		
Compartment	Sample name	fit in	fitting range (in eV or Å ⁻¹)	Cd-thiol or mixed/ amorph. CdS ^a	Cd-O	sum	Cd-thiol or mixed/ amorph. CdS	Cd-O	R factor ^b
grain	grain_Mat_I	XANES	-20 to 80	85	16	100	84	16	1.70E-02
straw	straw_Mat-III	XANES	-20 to 80	21	78	99	21	79	6.50E-03
	straw_Mat-II	XANES	-20 to 80	21	79	100	21	79	4.88E-03
	straw_Mat-I	XANES	-20 to 80	19	83	103	19	81	6.04E-03
shoot	shoot_Flow_I	XANES	-20 to 80	75	25	100	75	25	1.20E-03
		EXAFS	3 to 12	68	33	100	68	33	1.33E-01
node	node_Mat_II	XANES	-20 to 80	100	0	100	100	0	2.86E-03
		EXAFS	3 to 11	109	0	109	100	0	2.57E-01
	node_Mat_I	XANES	-20 to 80	100	0	100	100	0	1.64E-03
		EXAFS	3 to 10.5	103	0	103	100	0	5.54E-02
	node_Flow_I	XANES	-20 to 80	100	0	100	100	0	8.40E-04
		EXAFS	3 to 11	106	0	106	100	0	1.38E-01
node_Flow_II	XANES	-20 to 80	100	0	100	100	0	2.04E-03	
root	root_Mat_I	XANES	-20 to 80	100	0	100	100	0	7.49E-03
		EXAFS	3 to 11	109	0	109	100	0	3.52E-02
	root_Mat_Ia	XANES	-20 to 80	100	0	100	100	0	2.83E-03
		EXAFS	3 to 11	101	0	101	100	0	5.80E-02
	root_Mat_II	XANES	-20 to 80	100	0	100	100	0	1.72E-03
		EXAFS	3 to 11.7	93	6	99	94	6	4.11E-02
	root_Flow_I	XANES	-20 to 80	100	0	100	100	0	1.13E-03
		EXAFS	3 to 10.7	107	0	107	100	0	3.84E-02
soil	soil_Mat_II	XANES	-20 to 80	75	25	100	75	25	1.04E-03
	soil_Mat_I	XANES	-20 to 80	53	47	100	53	47	3.40E-03
	soil_Flow_I	XANES	-20 to 80	38	63	101	38	62	1.90E-03
		EXAFS	3 to 11	34	65	99	34	66	1.27E-01
	soil_Flow_Ia	XANES	-20 to 80	11	88	99	11	89	1.80E-03
	soil_Flow_II	XANES	-20 to 80	31	69	100	31	69	5.70E-03
		EXAFS	3 to 11	16	61	77	21	79	4.60E-01
	soil_initial_I	XANES	-20 to 80	13	88	101	13	87	5.40E-03
EXAFS		3 to 10	0	99	99	0	100	2.10E-01	

Flow: flowering stage. Mat: full maturity stage. I/II/III denote experimental replicates. "a" denotes analytical replicate. Reference compounds used as proxy for Cd-thiol or mixed/ amorph. CdS: Cd-metallothionein, Cd-phytochelatin, Cd-GSH, Cd-cysteine and (Cd, Zn, Fe)S. Reference compounds used as proxy for Cd-O: Cd-organic acid complexes, Cd phosphate, Cd phytate, and Cd sorbed on goethite and ferrihydrite.

Table_S 7: Dry weight and micronutrient concentrations in the upper shoot at maturity

compartment	period/ growth stage	DW		Zn			Mn			Ni		Cu							
		dry weight (DW)		concentration			mass			concentration		mass							
		g		$\mu\text{g g}^{-1}$	μg		$\mu\text{g g}^{-1}$	μg		$\mu\text{g g}^{-1}$	μg	$\mu\text{g g}^{-1}$	μg						
mean	$\pm\text{sd}^{\text{a}}$	mean	$\pm\text{sd}^{\text{a}}$	mean	$\pm\text{sd}^{\text{a}}$	mean	$\pm\text{sd}^{\text{a}}$	mean	$\pm\text{sd}^{\text{a}}$	mean	$\pm\text{sd}^{\text{a}}$	mean	$\pm\text{sd}^{\text{a}}$						
shoot		45.0	3.22	40.5	4.15	1575	109	239	14.7	9314	796	0.91	0.14	36	6.1	4.0	0.73	155	24.1
flag leaves	maturity	1.35	1.05 b	14.5	0.61 c	21.8	15.9 b	245	26.1 b	352	229 b	0.48	0.16 b	0.69	0.47 c	4.23	0.47 ab	6.49	5.02 b
panicle		2.81	0.69 b	46.7	2.48 a	132	39.3 a	358	21.3 a	1012	295 a	1.38	0.16 a	3.9	1.14 b	3.54	0.65 b	9.92	2.66 b
grains		4.83	1.01 a	26.9	1.24 b	130	25.6 a	26.1	5.26 c	125	30 c	1.60	0.35 a	7.75	2.35 a	4.78	0.69 a	23.0	5.70 a

^asd = standard deviation of the mean of n = 4 experimental replicates. Letters indicate significant differences of the mean determined using ANOVA (non transformation: DW, Zn mass; 1/x transformation: Cu conc.; sqrt transformation: Zn conc., Mn conc., Ni conc.; Ni mass; log10 transformation: Mn mass.)
^bthe sd was calculated using error propagation as described in equations 4-5.



Figure_S 1: Overview rice parts analyzed.

At flowering, the roots were separated from the shoots and then the nodes were separated from the shoot. At maturity, roots were separated from the shoot and the shoot was dissected into nodes, straw, and upper shoot. The latter was further dissected into flag leaves, panicle (rachis and spikelets), and grains. For both growth stages, the nodes sampled are defined as 'node I' according to Yamaji and Ma (2014).